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Biochimica et Biophysica Acta 1502 (2000) 31–43

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

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Review

Oligomerization and fibril assembly of the amyloid-β protein

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Received 6 August 1999; received in revised form 28 December 1999; accepted 28 December 1999

Abstract

In this chapter, we attempt to analyze the evolution of the amyloid-β (Aβ) molecular structure from its inception as part of the Aβ precursor protein to its release by the secretases and its extrusion from membrane into an aqueous environment. Biophysical studies suggest that the Aβ peptide sustains a series of transitions from a molecule rich in α-helix to a molecule in which β-strands prevail. It is proposed that initially the extended C-termini of two opposing Aβ dimers form an antiparallel β-sheet and that the subsequent addition of dimers generates a helical Aβ protofilament. Two or more protofilaments create a strand in which the hydrophobic core of the β-sheets is shielded from the aqueous environment by the N-terminal polar domains of the Aβ dimers. Once the nucleation has occurred, the Aβ filament grows in length by the addition of dimers or tetramers.

Keywords: Alzheimer's disease; AβPP; Dimer; Molecular structure; Membrane; Secretase

1. Introduction

The brains of patients with Alzheimer disease (AD) are characterized by the presence of large deposits of extracellular fibrillar amyloid-β (Aβ) peptide in neuritic plaques and in the walls of cortical and leptomeningeal vessels. The 10–12 nm diameter amyloid fibrils result from the polymerization of the 40–42 amino acids long Aβ peptide. The Aβ molecules are derived from the 695 amino acids long type-I transmembrane protein, known as Aβ precursor protein (AβPP), by the action of the β- and γ-secretases [1]. The Aβ is very reactive at physiological pH since it contains seven positive and seven negative charges, mostly localized at the N-terminal region of the molecule. The Aβ peptide also contains two hydrophobic domains comprising residues 17–21 and residues 29–40/42, this latter sequence corresponds to 12–14 amino acids of the 24 residues putatively representing the transmembrane domain of AβPP [1]. A third proteolytic enzyme, the α-secretase, cleaves the AβPP at residue 612, using the AβPP695 numbering. When this cleavage occurs in combination with the γ-secretase, the resulting peptide, Aβ residues 17–40/42, is apparently non-toxic [2], non-fibrillar and mostly confined to diffuse deposits scattered through-

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0925-4439/00/$ – see front matter
PII: S0925-4439(00)00030-2

out the neuropil [3,4]. Several studies have demonstrated that the Aβ isolated from the neuritic plaque cores is extensively truncated at the N-terminus, probably resulting from the proteolytic action of amino-peptidases [5–7]. This degradation is also observed, although to a lesser degree, in the amyloid of the cerebro-vascular deposits [8]. As a long-lived molecule, the Aβ peptide accumulates a series of post-translational modifications such as isomerization of Asp-1 and Asp-7 [7,8], racemization of Asp-1, Asp-7, Asp-23 and Ser-8 [7,9,10], cyclization of Glu-3 and Glu-11 [11–13] and oxidation of Met-35 [5,14]. These modifications render the Aβ fibrils more stable, insoluble and resistant to proteolytic degradation [14,15]. For reasons still unclear, most of the Aβ present in the fibrillar deposits of the neuritic plaque cores corresponds to the Aβ42 type, while the parenchymal and leptomeningeal vascular deposits have a higher proportion of the more soluble Aβ40 [7,8,16–20]. Interestingly, the amount of Aβ40 is excessively accumulated in the cerebral vasculature of those individuals with AD carrying the apolipoprotein (Apo) E genotype ε4/ε4 [21–24].

Until recently, the fibrillar Aβ40/42 was considered to be the only toxic form of this peptide [25,26]. The profuse deposition of fibrillar Aβ in the neuritic plaques of the cerebral cortex appears to be associated with dystrophic neurites [27]. Surrounding the spherical cores of amyloid reactive microglia and astrocytes appear to participate in the production of fibrillar Aβ and in the insulation of this material from the rest of the neuropil [28]. The discovery of a pool of soluble amyloid in AD brains and in cultured cells triggered a reassessment of the role of monomeric/dimeric and oligomeric Aβ in the pathology of this dementia [29–32]. Damage to neurons may be caused by intracellular and, to a lesser extent, extracellular highly reactive and toxic Aβ dimers and oligomers that can easily diffuse inside the cells and throughout the narrow and convoluted extracellular space of the brain. As a mechanism of defense against this perturbation, the glial cells apparently participate in the efficient uptake of obnoxious soluble extracellular Aβ and in the 'dumping' of this material at localized sites where the Aβ is concentrated. Soluble Aβ40/42 appears to be universal in physiological fluids such as cerebrospinal fluid, plasma and urine of AD and normal individuals [29,33–36].

In this chapter, we attempt to analyze the evolution of the Aβ molecular structure from its initial inception as part of the AβPP to its release by the secretases and its extrusion from the membrane into an aqueous environment. Biophysical studies suggest that the Aβ peptide sustains a series of transitions from a molecule rich in α-helices to a molecule in which β-sheets prevail. It has been proposed that dimerization and oligomerization generate a nucleus prompting the creation of stable filaments to shield hydrophobic domains and overall to reduce entropy.

2. The initial membrane–Aβ topology

To understand the process of AβPP proteolysis by the α-, β- and γ-secretases and the ensuing release of the short (Aβ17–42) and the long (Aβ1–40/42) Aβ peptides, it is necessary to investigate the conformational relationships between these domains and the membrane. The actions of the α- and β-secretases on the AβPP molecule may be influenced by the local membrane environment which in turn is dictated by the lipid composition of the bilayer. Among these molecules, the well-regulated amount of cholesterol inserted in the membranes appears to largely contribute to the thickness and fluidity of these structures [37,38]. Different membranes, whether cytoplasmic, endoplasmic, Golgi, mitochondrial, lysosomal, contain variable quantities of cholesterol and other lipids intimately linked to their specific functions [39,40]. The length of hydrophobic transmembrane domains of proteins residing in different organelles correlates with their cholesterol content [39,41]. In addition, specialized sub-domains within membranes, such as caveolae and rafts, are also likely to influence the function and structural association between membrane and intrinsic proteins [42–44]. Differences in cholesterol concentration in different membranes are sufficient to regulate transmembrane insertion and membrane protein folding [45,46]. The role of cholesterol in AβPP processing was recently underlined by the observation that in tissue culture, the addition or depletion of this lipid results in significant variations in the production of Aβ or processing of the AβPP [47–50]. This relationship between AβPP, Aβ and cholesterol may be relevant to AD since amounts of total brain cholesterol have also

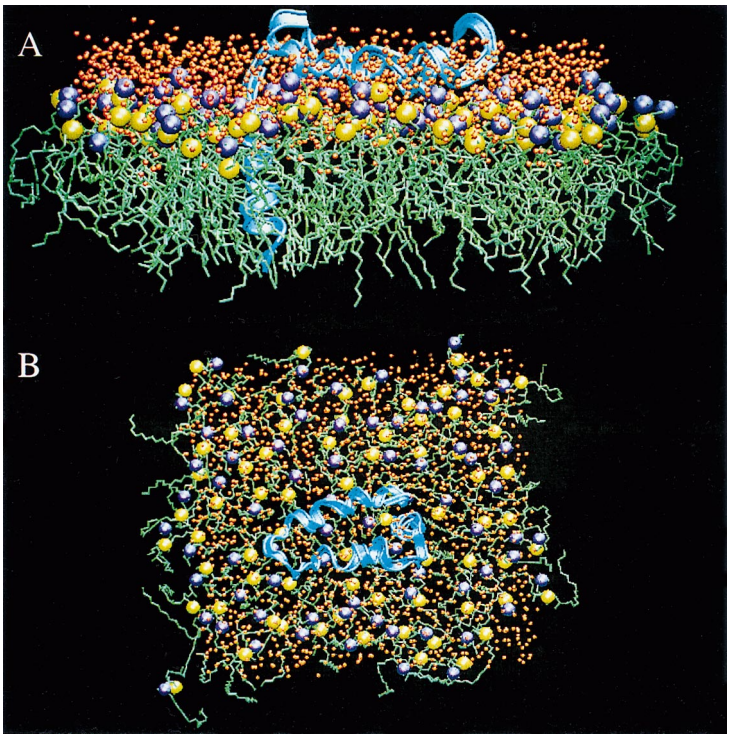


Fig. 1. Molecular modeling of the AβPP fragment (corresponding to the Aβ sequence of residues −12–42) inserted in a lipid membrane monolayer. (A) Side view. (B) Top view. The peptide is represented as a blue ribbon and the solvating water oxygens are depicted as red spheres. The phosphorus and nitrogen atoms of the lipid molecules are drawn as yellow and blue spheres, respectively. The long hydrocarbon chains of the lipids are represented in green. This structure will be used in a future study as the starting point for molecular dynamics simulations at room temperature. These simulations will address the important question of the relative angle between the two surface α-helices, containing the α- and β-secretase sites and the possible modification of this angle upon insertion of cholesterol in the membrane.

been found to be altered in AD when compared to controls [51–53].

As an initial attempt to model the possible topographical relationships between membrane and the Aβ peptide, a fragment of the amyloid precursor protein (AβPP fragment: −12–42) was selected:

NIKTEEISEVKMDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

-12 -1 1 42

This peptide, containing the Aβ sequence 1–42 plus 12 additional amino acid residues immediately adjacent to the N-terminus, was modeled and inserted into a lipid monolayer paradigm. The molecular modeling software QUANTA, version 98.1111 (Molecular Simulations Inc., San Diego, CA, USA) was used for molecular constructions and energy minimizations. The force field parameters of CHARMm version 25 [54] were used in the calculation. The three-dimensional structure of this fragment was determined by secondary structure prediction methods using the MPSSP secondary structure module within the Biology Workbench [55]. The consensus structure indicated a propensity for α-helices comprising residues: −7–5, 10–22 and 29–42, with turns in between (see Figs. 1 and 2). The AβPP fragment was manually inserted in a hydrated lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine monolayer, derived from the bilayer model developed by Heller et al. [56]. The procedure of building the protein–lipid complex involved deleting one lipid molecule from the monolayer model, and inserting the hydrophobic C-terminal α-helix residues 29–42 into the membrane cavity. The molecular structure was energy-minimized using the Adopted-Basis Newton–Raphson algorithm until the rms force was below 0.1 kcal/mol/Å. The compositional structure is shown in Figs. 1 and 2. The figures were produced using the program VMD [57].

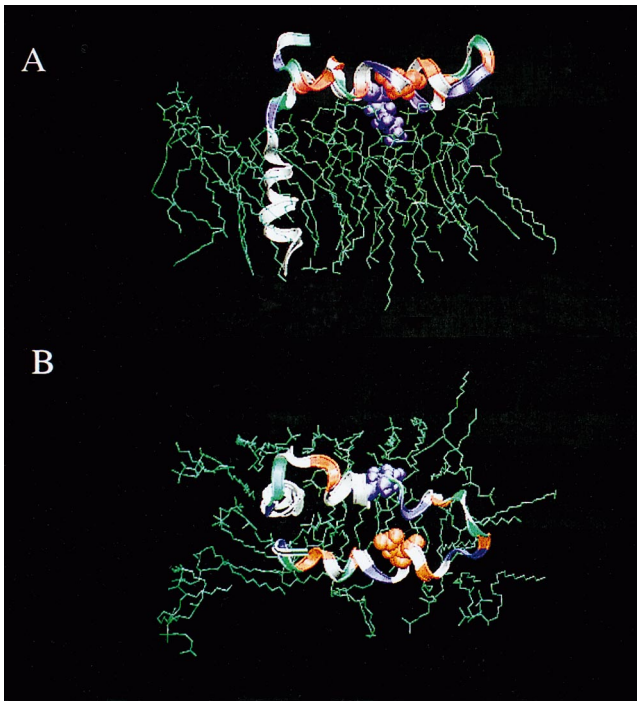


Fig. 2. Molecular modeling of the AβPP fragment (same model as in Fig. 1) represented as a ribbon. Only lipids close to the peptide are drawn in green color. (A) Side view. (B) Top view. The positions of the acidic and basic amino acid residues are shown in red and blue colors, respectively. Green and white denote the positions of polar and non-polar residues. In the model, the positions of the lysyl 16 and aspartyl 1 (blue and red van der Waals spheres, respectively), the sites of the α- and β-secretase postulated activity, are shown in close proximity, lying along the antiparallel α-helices.

The AβPP fragment–membrane monolayer model is useful to investigate the disposition of lumenal α-helices (residues −7–5 and 10–22) relative to the membrane surface. The model suggests a corner (residues 23–28) at the lumenal side of the transmembrane C-terminal sequence of residues 29–42, placing the two N-terminal α-helices parallel to the membrane surface. In addition, the existence of a second loop at residues 6–9 positions the two N-terminal α-helices in an energetically favorable antiparallel orientation, thereby stabilizing their interactions. On the membrane surface, some of the phosphate groups and nitrogen atoms of the lipid molecules will be in close proximity to interact with the positively charged (−2K, 5R, 6H, 13H, 14H and 16K) and negatively charged (−8E, −7E, −4E, 1D, 7D, 11E, 22E and 23D) groups of the AβPP fragment. Interestingly, in this preliminary model, there is a close proximity between lysyl 16 and aspartyl 1, the sites of the α- and β-secretases, respectively. Moreover, the ε-amino group of the former appears to be oriented towards the membrane surface whereas the β-carboxyl of the latter has a more superficial orientation (Fig. 2). Some studies have suggested that the α-secretase cleavage of AβPP is not sequence specific, rather the distance from the surface of the adjacent membrane, corresponding to 12 amino acids, appears to be the determinant factor [58,59]. This interpretation does not necessarily mean a distance in terms of Å from the Aβ residue 28 to the Aβ residue 16, but in all probability is related to the conformational properties of the surrounding structures in relation to the membrane surface. In synthesis, the angle of orientation between the two α-helices relative to each other and to the surface of the membrane would be dictated by a complex field of non-polar and ionic interactions. The thickness of the membrane will influence these interactions by modifying the position of the transmembrane domain, and of the surface helices relative to the interfacial environment. We propose that thicker membranes, caused by the addition of cholesterol, will result a deeper penetration of surface-located helices within the membrane interface [60]. This shift in membrane–AβPP topology may result in steric hindrance of the α-secretase site and render the β-secretase site more accessible to proteolysis and consequently to the increased production of Aβ peptides.

The determination of the Aβ structure by nuclear magnetic resonance (NMR) or by X-ray diffraction analysis in aqueous buffers under physiological conditions has been fraught with frustrations because of the propensity of the Aβ peptides to aggregate. However, some valuable NMR structural information has been obtained by dissolving the Aβ1–40 peptide in 40% trifluoroethanol/water [61]. Under this condition, the secondary structure of Aβ reveals the conformation of a random coil and two helices comprising the sequence of residues 15–23 and 31–35. More recently, the NMR structure of Aβ1–40 was determined in a water–sodium dodecyl sulfate (SDS) micelle medium which is analogous to a water–membrane environment [62,63]. The peptide appears to be unstructured and water-solvated between residues 1 and 14, followed by a long α-helical sequence from residues 15 to 36, clearly solvated by the SDS, inter-

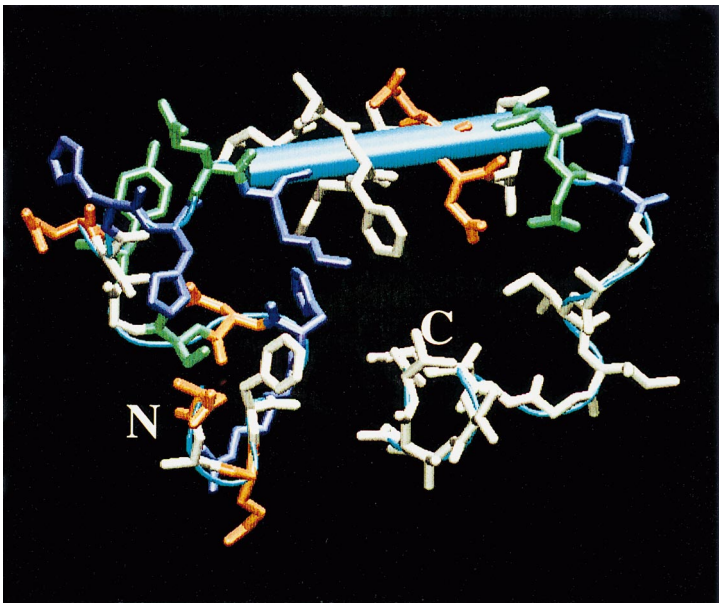


Fig. 3. The structure of the Aβ1–40 peptide (35 methionyl sulfoxide) in water–SDS micelle medium obtained by NMR [63]. The only α-helical segment comprising residues 16–24 is indicated by the blue rod. N and C indicate the N- and C-termini, respectively. The amino acid residues side chain characteristics are identified by the same color code as given in Fig. 2.

rupted by a hinge around residues 25–27. With increasing pH, in the water–SDS micelle environment the Aβ1–40 undergoes a structural transition from α-helix to random coil probably related to the deprotonation of residues 22E and 23D. In addition, the accepted location of the transmembrane domain (residues 29–52) has been challenged by Craik and colleagues who suggested the sequence of residues 25–46 instead [62]. This is an appealing concept since the site of the γ-secretase has been suggested to be residue Aβ46 [64] and is also the site of several AβPP mutations (V46F, V46G and V46I) observed in familial AD [65–67]. Shortening of the C-terminus of Aβ1–46 to either residue 40 or residue 42 could be efficiently performed by carboxypeptidases.

The molecular structure of Aβ1–40 in water–SDS micelle medium, with the methionyl at position 35 oxidized by hydrogen peroxide to yield methionyl sulfoxide, was recently investigated by circular dichroism (CD) and NMR spectroscopy [63]. Under these conditions, the Aβ peptide adopts a random coil structure with one helical region comprising residues 16–24. Other NMR studies performed at different pHs and solvent conditions have also revealed a helical structure around this sequence [61,68]. In Fig. 3, we recreated the three-dimensional structure of the oxidized 35 methionyl Aβ1–40 as deposited in the Protein Data Bank (1ba6). Aβ probably exists in a dynamic flux of different conformations, dependent upon environmental conditions [69–71], interactions with other molecules, and metal binding ability [72–74]. The mostly unstructured molecule shown in Fig. 3 may represent a transitional conformation along the accepted evolution of Aβ from α-helix → random coil → β-sheet [75]. Interestingly, the stable α-helical sequence of residues 16–24 (KLVFFAEDV) apparently plays an important role in fibrillogenesis. Substitutions in the hydrophobic sequence of residues 17–20 by polar amino acids decrease the β-sheet content in water and in the solid state [76]. Replacement of any residue within the sequence of residues 17–23 by prolyl increases the solubility of Aβ and alters the stability of the β-sheet inhibiting filament formation [77]. Furthermore, in three independent studies, addition of the synthetic peptides KLVFF (Aβ residues 16–20), or KLVFFAEDVG (Aβ residues 15–25) or LPFFD (based on the Aβ sequence LVFF) to solutions of freshly prepared synthetic Aβ partially inhibited fibrillogenesis [78–80]. It is possible that the basic building block of the Aβ filaments is the dimer-

ic Aβ. We proposed in our previous model that the hydrophobic sequence of residues 17–21 is pivotal in the process of dimerization [81].

The soluble monomeric largely amphoteric and amphipathic Aβ is very reactive with a very large number of molecules as already demonstrated by several laboratories [82–94]. The presence of free Aβ in the brain and physiological fluids of non-demented individuals suggests that either the Aβ levels are too small to cause perturbations or alternatively, the peptide exists in a non-toxic conformation or is efficiently cleared. The role of chaperone or carrier molecules may be decisive in maintaining the latter conditions.

3. From Aβ monomers to polymeric fibrils: experimental observations and models

The release of the Aβ peptide from its associated membrane and AβPP results in its exposure to a thermodynamically unfavorable aqueous medium and to interactions with ions and multiple molecules including another Aβ peptide. Recent data have revealed the presence of stable soluble dimeric Aβ in brain homogenates [95–97], cell culture media [98] as well as in synthetic Aβ preparations [14,99,100]. Likewise, Aβ may readily associate with other numerous molecules such as α1-antichymotrypsin [85,86], albumin [89,90], Apo E [101,102], Apo J [82,103], α2-macroglobulin [84,104], amyloid P component [105,106], complement proteins [91,92], α-synuclein [107,108], heparan sulfate proteoglycan [87,109], receptor for advanced glycation end-products [93], endoplasmic reticulum Aβ binding protein [94,110], just to name a few. In the final analysis, the structural changes and molecular interactions experienced by the Aβ peptide in the human brain, from the moment of its membrane release to its final incorporation into the extracellular amyloid filaments, still remain shrouded in mystery. Examination of the AD cerebral cortex by electron microscopy demonstrated that the extracellular amyloid deposits form an array of ~10 nm filaments of a variable length [27,111]. In cross-section, the filaments appear to be composed of a series of 5–6 electron dense symmetric subunits peripherally disposed around an electron-translucent core [112,113]. X-ray diffraction analysis of Aβ filaments revealed a predominant feature in which there are meridional reflections at 4.76 Å and equatorial reflections at 10.6 Å. These reflections suggested a model in which a series of antiparallel cross-β pleated sheets are oriented parallel to the main axis of the filament, while the direction of the constituent β-strands is perpendicular to the fibril axis, making the hydrogen bonds between strands parallel to the fibril axis [114,115].

Experiments with synthetic Aβ peptides showed that lyophilized Aβ when dissolved in aqueous buffers sustains a series of conformational transitions. Infrared spectroscopy suggests that these changes may be initiated by the generation of antiparallel β-strands joined by a hairpin loop formed by residues 26–29 [116]. CD of Aβ dissolved in aqueous buffers indicates the presence of two populations in terms of secondary structure: one with an extensive random coil conformation and another with predominant β-strands [75,117]. Short term incubation promotes the transition of the latter species of Aβ into filamentous structures [75]. Long term incubation of synthetic Aβ1–42 results in the intrafibrillar generation of irreversibly denatured dimers and tetramers that are highly resistant to proteolytic degradation [14]. Furthermore, the irreversible oligomerization is accelerated by the presence of aspartyl isomerizations which are commonly observed in the Aβ isolated from the amyloid cores of the neuritic plaques [14].

Molecular modeling has been used to predict the three-dimensional structure of Aβ1–42 [81]. This study was based on sequence homology, the threading algorithm TOPITS [118] and experimental observations derived from numerous biophysical observations. The structure of Aβ1–42 was optimally superimposed on the α-carbon backbone of three TOPITS hits represented by the lipid binding proteins: 1ifb, 1pya and 1cid [81]. These three molecules exhibited a common antiparallel β-structure with Aβ. Because of their length and the torque imposed by the hydrogen bonding, the initial antiparallel β-strands may promote the generation of a Greek key motif [119–121]. This structural motif displays four antiparallel β-strands with corners formed by the Aβ residues 7–8, 16–17, 25–28 and 37–38. In order to shield unfavorable hydrophobic domains from the aqueous media, the Aβ probably interacts with another molecule to form a dimeric structure

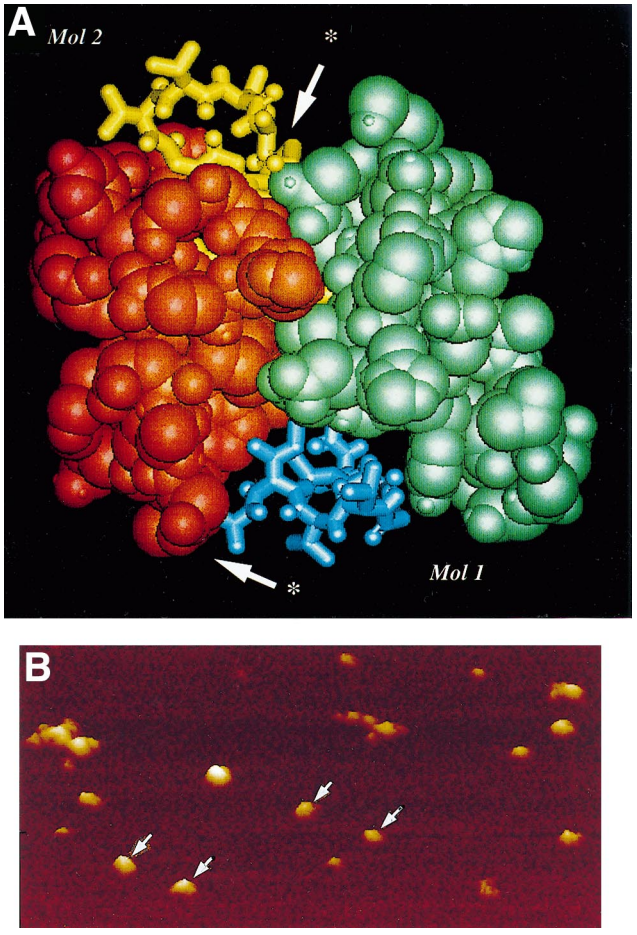
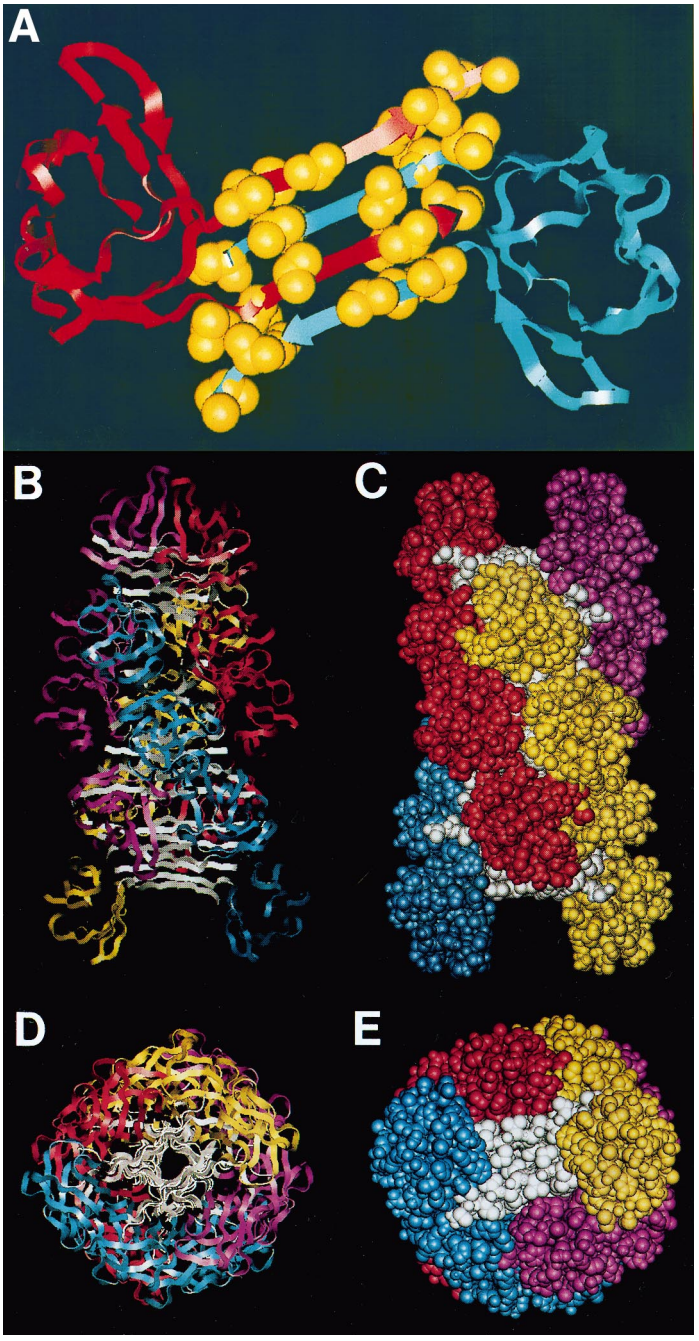


Fig. 4. A model and AFM of Aβ dimers. (A) A low energy three-dimensional model of dimeric Aβ. A detailed description of the physicochemical characteristics of the model is given in a previous publication [81]. The amphipatic N-terminal regions (residues 1–28) of each of the Aβ monomers are shown in red and green space filling display. Each of the hydrophobic C-terminal domains, shown in yellow and blue licorice, folds into shallow crevices formed by the mostly polar N-terminal regions. The Aβ dimer generates a compact globular structure which measures 27 Å by 32 Å. The arrows indicate the point at which the C-terminus of one Aβ molecule interacts with the N-terminus of the adjacent Aβ molecule. (B) AFM views of dimeric Aβ isolated from the neuritic plaque cores of amyloid separated by column chromatography. The arrows point to the dimeric peptide which measured 35–38 Å. The larger diameter of the AFM dimer as against that of the theoretical model may be due to the hydration shell around the human-derived material. The molecular mass of Aβ dimers was confirmed by mass spectrometry and Western blot [95].

with the conformational characteristics of a β-barrel. Abnormalities in side chain interactions and carbon backbone exertions were relieved by means of molecular dynamic simulations and energy minimizations. The dimeric Aβ structure displays a hydrophobic core surrounded by hydrophilic residues creating shallow crevices into which the non-polar C-termini are folded (Fig. 4A). The compact globular dimeric Aβ1–42 structure measures ~27 Å×32 Å similar in dimensions to the Aβ dimers isolated from the AD brain (~35 Å) as measured by atomic force microscopy (AFM) which is shown in Fig. 4B [95]. The dimeric Aβ model has the positive and negative charges at the surface and exposes the sequence of residues 13–16 (HHQK). This sequence is critical in the interaction of Aβ with the heparan sulfate proteoglycans on the surface of microglia eliciting in these cells the ability to kill neurons [2,122]. The dimerization of Aβ also creates a potential field for zinc binding [72]. In general, the conformational features of the N-terminal domains in the Aβ dimers also agree with other physicochemical studies in terms of Aβ topology, aggregation and toxicity [87,123–127].

In the designed model, the hydrophobic C-termini (residues 30–42) by virtue of being at the surface of the dimeric structure have the ability of extending and interacting with the equally extended C-termini of an opposite dimer to create an antiparallel β-sheet [81]. This structure, shown in Fig. 5A, was based on the solid state NMR studies of Lansbury and colleagues in which the C-terminal sequence of residues 34–42 naturally produced an antiparallel stack of β-strands [128]. The adjacent addition of these stacks will create a ribbon-like helical structure, a protofilament, similar to that described for the transthyretin by Blake and Serpell [129]. The amphipathic and amphoteric N-terminal domains of Aβ, comprising residues 1–28, are localized at opposite sites interacting with the N-termini of adjacent Aβ dimers. During the transition from dimer to protofilament, the sequence of residues 17–23 re-localizes on the opposite interfaces of the Aβ dimer potentially interacting with supra- and infra-adjacent dimers. In terms of energy, the protofilament is thermodynamically in an unfavorable condition because the hydrophobic β-sheet is exposed to the aqueous media. To overcome this situation, two or three nascent protofilaments may associate side by side to create a nucleus of an intertwined helical structure in which the nonpolar C-terminal sequences are shielded by the mostly hydrophilic N-terminal domains of Aβ [81].



A molecular model of two helical protofilaments interwined side by side is shown in Fig. 5B–E. Based on transmission electron microscopy [130] and AFM studies [95,131,132], two of these putative strands may be further intertwined to generate an 8–10 nm Aβ filament similar to those observed in the AD brain.

In an alternative model, the Aβ residues 14–23 have been proposed as the basic sequence in fibril formation because of its capacity for generating antiparallel β-sheets with the same sequence of adjacent Aβ molecules [133]. The hydrophobic C-terminal sequence of Aβ (residues 30–42) is proposed to form an intramolecular β-sheet that folds over the core of

Fig. 5. Molecular models of Aβ dimers and protofilaments. (A) Two Aβ dimers with extended C-termini forming an antiparallel β-sheet stabilized by intermolecular hydrogen bonding and hydrophobic interactions. This model is based on the NMR studies of Lansbury and colleagues [128]. The β-sheet is flanked by the N-terminal globular domains of the dimers (represented in red and blue ribbons). The successive stacking of dimers creates a helical ribbon or protofilament. A model of a two-protofilament strand is shown in ribbons and space filling displays viewed in a normal to the filament axis (B and C) and along the filament axis (D and E), respectively. Each protofilament resembles a helical 'ladder' in which each of the 'railings' is formed by the consecutive superimposition of the N-terminal domains (residues 1–28) of Aβ dimers (shown in yellow and blue, and red and purple), and the 'rungs' formed by the extended C-termini antiparallel β-strands (residues 30–42) of opposing Aβ dimers (depicted in white). Two protofilaments intertwined in parallel against each other generate a core of two helical β-sheets (white ribbons and balls) with their hydrogen bonds parallel to the protofilament main axis. In this structure, the hydrophobic C-termini of the Aβ peptides are shielded from the aqueous environment by the mostly polar N-terminal Aβ residues. For simplicity, a strand made of two protofilaments is shown. However, models with strands of three [81] and four protofilaments can also be generated. We suggest that, in vivo, the accretion of Aβ molecules at the growing end of the filament is in the form of either dimers or tetramers rather than by the lateral assembly of preformed protofilaments. A is reproduced with permission from Oxford University Press [81].
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residues 14–23. In this model, the N-terminal domain of Aβ residues 1–13 was considered non-important in the process of fibrillogenesis since it is partially degraded in the neuritic plaque core amyloid. However, partial degradation of the N-terminus is insignificant in vascular amyloid [7,8]. We believe that in Aβ fibrillogenesis, as it occurs in the brain, the N-terminal region of this peptide is essential to shield the C-terminal hydrophobic core [81]. In all probability, in the neuritic plaque amyloid deposits the N-terminal degradation of Aβ occurs after the fibril is forged.

Several experimental observations have demonstrated the in vitro presence of Aβ protofilament intermediates [100]. By size exclusion chromatography, synthetic Aβ1–40 dimers elute, after 24 h of incubation as a single fraction. A wide range of Aβ protofilaments, measuring 6–10 nm in diameter and 5–160 nm in length, are eluted as a single fraction in the void volume [100]. In this experiment, fully grown Aβ filaments were sedimented by centrifugation prior to the chromatographic step. Those remaining in the supernatant too large to transit in the void volume of the matrix are retained at the top of the column. We would like to suggest that the protofilaments fractionated by column chromatography may represent part of continuous distribution of actively growing filaments more than an intermediate and discrete subclass of aggregates. If indeed the neurons are the major source of Aβ in AD, then electron microscopy has failed to localize protofilaments, in the range of sizes observed in the chromatographic void volume, either in the neuronal cytosol or organelles.

The absence of intraneuronal protofilaments and filaments suggests that in these cells, damage may be done by soluble Aβ monomers/dimers or very small oligomers. Soluble Aβ is a very reactive molecule due to its ability to establish electrostatic and hydrophobic interactions. It is capable of rapidly binding to a large number of molecules and membrane structures causing serious alterations in cellular homeostasis, and the neuron should not be an exception [94,134–140]. The available evidence suggests a scenario in which Aβ as soon as it is released from AβPP and extruded from the membrane reacts with other molecules hindering fibrillogenesis. Following secretion of Aβ complexes and uptake by glial cells, the toxic soluble Aβ is finally deposited in a localized area. Fibrillogenesis may developed by a nucleation dependent association and growth by the end-terminal addition of Aβ to yield a helical fibrillar polymer [141]. It has been observed that deposition of Aβ onto pre-existing templates of amyloid follows first-order kinetics and is dependent on the Aβ concentration with an optimum at physiological pH [142]. In addition, the narrow and intricate extracellular space of the brain parenchyma filled with extracellular matrix would hinder the diffusion of protofilaments. It has been proposed that the glial electron-translucent coated vesicles, putatively carrying non-filamentous Aβ, fuse with the cell membrane to form coated pits where the polymerization of amyloid filaments is suggested to occur [143]. A similar situation has been observed in the transgenic human AβPP mice [144]. A related phenomenon may take place at the surface of the vascular tunica media where soluble Aβ complexed to carrier molecules is

internalized by myocytes and subsequently deposited along the extracellular space [145]. In both cases, brain parenchyma and vascular walls, high concentrations of Aβ are secreted into defined extracellular microenvironments where fibrillogenesis takes place. The deposition of amyloid in the neuritic plaques by glial cells may be very similar to the deposition of procollagen into the cytoplasmic recesses of fibroblasts [146]. A recreation of Aβ oligomerization and fibrillogenesis in vitro probably have very little resemblance to the oligomerization and fibrillogenesis occurring in the highly complex environment of the human brain. This is illustrated by the intimate association of lipids, carbohydrates and proteins with the amyloid fibrils purified from the AD brain [147]. Based on high resolution electron microscopy, a model of the neuritic plaque amyloid filaments has recently been proposed by Kisilevsky and colleagues [148]. In this paradigm, a core of amyloid P component and chondroitin sulfate proteoglycan is surrounded by a layer of heparan sulfate proteoglycan onto which a peripheral coat of 1 nm Aβ filaments is associated.

Fibrillar Aβ is beyond doubt a toxic and extraneous material capable of triggering and sustaining a subtle but chronic inflammatory reaction [149] that further contributes to the destruction of the neuropil. However, a collection of polymerized Aβ at the center of the neuritic plaque surrounded by glial cells may be the most efficient way of concealing, concentrating and immobilizing neurotoxic soluble Aβ. This tenet is well-illustrated by our recent finding that the cortical soluble Aβ concentration is the best predictor of synaptic change in the brain of AD patients [150]. In addition, overexpression of mutant human AβPP in neurons of transgenic mice causes a decline in the density of presynaptic terminals and neurons well before amyloid plaques appear [151]. Both of these observations suggest that soluble Aβ, free or in association with other molecules, is capable of injuring the neurons. Finally, it would not be farfetched to suggest that in the absence of localized fibrillar Aβ deposits, the widespread distribution of toxic soluble Aβ may result in a shorter clinical evolution of AD. In this context, we also suggest that the cores of amyloid in the AD brain may represent a mechanism of defense, which in the end also has catastrophic consequences.

Acknowledgements

This study was partially supported by The National Institutes of Health (AG-11925 and 5P41-RR05969), by The National Science Foundation (BIB 94-23827Eq; BIR 93-18159), by The Roy J. Carver Charitable Trust, by The Beckman Institute for Advanced Science and Technology, University of Illinois, Urban-Champaign, and by the State of Arizona Center for Alzheimer Disease Research.

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